INCREASE OF REACTIVE GROUPS IN THE SURFACE OF PLGA PARTICLES BY STABILIZATION WITH POLYAMINOACIDS

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Drug-loaded carriers with optimal targeting properties are an actual pharmaceutical drug delivery requirement. The possibility to administrate a carrier with specific attachment to concrete cells or organs in the body would increase the drug safety by minimizing systemic size effects.

A commonly used biomaterial in new drug delivery systems is poly (lactic-co-glycolic acid) (PLGA) [1]. The attachment of ligands to PLGA based carriers for their site specific delivery is limited by their lack of chemical reactivity. Moreover, although PLGA with end-carboxylic groups are commercialized, PLGA micro- or nanoparticles are currently stabilized by block copolymers, such as poly (vinil alcohol) (PVA) or poloxamer, that stick to the surface of particles hindering any functional groups.

Strategies used in order to increase the reactivity of PLGA polymers, as copolymerization with chemical containing functional groups, involve changes in the main chain structure of the polymer, which may change its biodegradability[2][3]. In this work, we proposed the replacement of the stabilizer PVA with others, able to provide themselves amine or carboxylic reactive groups to the surface of PLGA particles, and keep the main-chain structure of the polymer. For this purpose, we report the use of cationic (polyarginine) or anionic (polyglutamic acid) polyaminoacids.

PLGA particles were prepared by two traditional procedures, double-emulsion or nanoprecipitation. In the w/o/w emulsion method, polymer was dissolved in dicholomethane (200 mg in 5 mL). Next 1 mL double-distilled water was added to the organic polymer solution and both phases were emulsified using a microtip probe sonicator (20W energy output) for 1 min over ice bath. The primary emulsion was transferred to 20 mL of poly amino acid solution (different concentrations from 1% to 0.025%) for 2 min homogenization. The emulsion thus formed was stirred for 3h at room temperature to evaporate the organic solvent. When nanoprecipitation was performed 10 mL of acetone-polymer solution were added under soft magnetical stirring to 20 mL of a poly amino acids solution (concentrations 0.1% to 0.3%). Organic solvents were evaporated under reduced pressure.

Size and zeta potential of the particles was determined by photon-correlation spectroscopy (PCS). Large particles were sized by laser diffractometry (Malvern Instruments). Table 1 summarizes the results of PLGA particles prepared by double emulsion method in presence of PVA or polyglutamic acid as stabilizers. As shown, PVA stabilized PLGA particles were >1µm size. However, nanoparticles were obtained in presence of polyglutamic acid. Size decreased with increasing amounts of polyaminoacid until 0.1%. Higher concentrations resulted in polydisperse systems with two populations. With the nanoprecipitation method, monodisperse systems of 173 ±49 nm were obtained with 0.1% polyglutamic (data not shown).

Determination of carboxylic groups involved an activation with carbodiimide followed by the attack of nucleophile L-cysteine and determination of SH- groups with Ellmann

reaction[4][5][6]. As expected, the number of carboxylic groups rised with the concentration of polyglutamic acid whereas it decreased with the increase of PVA. By a similar technical procedure, polyglutamic stabilized particles were conjugated with mannosamine. Although the quantification of the sugar attached to the particles surface (carried with the resorcinol-sulfuric acid method) confirmed the functionalization of PLGA nanoparticles with mannose moieities, a phenomenon of aggregation happened during the process of activation-linkage. The resulting particles of around 12 μm cannot be phagocyted by antigen presenting cells, as dendritic cells. As long as our main aim is the use of these particles as antigen carriers for vaccination, experimental conditions are being reconsidered in order to obtain functionalized particles with a suitable size.

References:

[1] J. Pan, Y. Wang, S. Qin, B. Zhang, Y. Luo, Grafting reaction of poly(D,L)lactic acid with maleic anhydride and hexanediamine to introduce more reactive groups in its bulk. J Biomed Mater Res B Appl Biomater 74(1) (2005) 476-480.

[2] T.M. Fahmy, R.M. Samstein, C.C. Harness, W. Mark Saltzman, Surface modification of biodegradable polyesters with fatty acid conjugates for improved drug targeting. Biomaterials 26(28) (2005) 5727-5736.

[3] L.A. Dailey, M. Wittmar, T. Kissel, The role of branched polyesters and their modifications in the development of modern drug delivery vehicles. J Control Release 101(1-3) (2005) 137-149.

[4] L. Nobs, F. Buchegger, R. Gurny, E. Allemann, Surface modification of poly(lactic acid) nanoparticles by covalent attachment of thiol groups by means of three methods. Int J Pharm 250(2) (2003) 327-337.

[5] Y. Mo, L.Y. Lim, Mechanistic study of the uptake of wheat germ agglutinin-conjugated PLGA nanoparticles by A549 cells. J Pharm Sci 93(1) (2004) 20-28.

[6] M.J. Montisci, G. Giovannuci, D. Duchene, G. Ponchel, Covalent coupling of asparagus pea and tomato lectins to poly(lactide) microspheres. Int J Pharm 215(1-2) (2001) 153-161.

[Table 1] Physico-Chemical characterization of particles in the presence of PVA and Polyglutamic acid

STABILIZERS	**CONCENTRATION** (%)	**SIZE ± SD** (nm)	**PDI**	**CARBOXILIC GROUPS** (μmol/particles mg)	**SIZE (μm)** (after mannosamine attachment)
POLYGLUTAMIC ACID	1	3800	-	-	-
	0.5	238 ± 20.8 (60%) 7000 (40%) [a]	0.49	103.4	-
	0.25	312 ± 134 (80%) 5000 (20%) [a]	0.52	66.9	-
	0.1	184 ± 3.2	0.29	10.9	12 ± 20
	0.05	167 ± 2.4	0.26		19 ± 15
	0.025	676 ± 21	0.43		22 ± 16
PVA	5	no m	-		-
	2.5	no m	-		-
	1	no m	-		-
	0.5	1520 ± 300 [b]	-	12.9	-
	0.25	2250 ± 230 [b]	-	18.9	2 ± 0.5
	0.2	1840 ± 210 [b]	-	37.9	2 ± 0.5

PDI: polydispersity index

[a]Two particle populations, the percentage in number is shown in brackets

no m: no measurable, no particles formation

[b] D[3,4]: volume moment mean of the particle measured by laser diffractometry